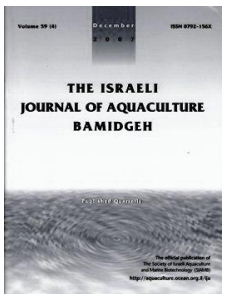
The Israeli Journal of Aquaculture - Bamidgeh, IJA_67.2014.990, 9 pages



Effects of processing method and dietary lysine levels on growth performance, feed conversion ratio and body composition of grass carp, *Ctenopharyngodon idella*

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Key words: grass carp, *Ctenopharyngodon idella*, lysine, processing method extruded pellets

Abstract

Effects of feed processing method and lysine level on the growth performance of grass carp were evaluated in this study. 13 g kg-1, 15 g kg-1 and 17 g kg-1 lysine level of diet were prepared through (a) extrusion and (b) cold pelleting process, supplemented with crystalline lysine (L-lysine HCL). After 8 weeks of feeding experiment, the results showed that the feed processing method had no effect on growth and on the feed conversion rate of grass carps. Weight gain, protein and amino acids retention, feed efficiency of grass carp fed with the extruded and pelleted feeds were significantly improved by with lysine supplementation ($P < 0.05$). Viscerasomatic index (VSI), hepatopancreasomatic index (HSI), intraperitoneal fat ratio (IPF), muscle fat content, whole body fat content, serum high density lipoprotein and low density lipoprotein of fish were significantly increased when fed extruded diets ($P < 0.05$). This result suggests that extrusion can affect fat metabolism. VSI, HIS IPF, muscle fat content and whole body fat content of grass carp were significantly reduced with supplemented crystalline lysine ($P < 0.05$). Lower serum Aspartate aminotransferase activity and glutamate dehydrogenase activity were observed when fish were fed with extruded diets ($P < 0.05$).

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Introduction

Extrusion processing is a technology commonly used in the aquaculture feed industry. Extruded diets are more durable, and have superior water stability compared to cold pelleted diets (Hilton et al., 1981; Yoshitomi, 2004; Misra et al., 2002). Some researchers have demonstrated that extrusion reduces phytic acid, gossypol and aflatoxin toxicities concentration of plant protein resources (Buser & Abbas, 2001; Satoh et al., 1998). Extrusion processing also can increase apparent starch digestibility (Venou et al., 2009). Extrusion can lead to Maillard reactions, resulting in a bioactivity loss of L-lysine and other susceptible amino acids, possible reducing dietary protein utilization (Hilton et al., 1981; Vens-Cappell, 1984). When fish were fed extrdued diets, feed efficiency was improved (Akimoto et al., 1992; Booth et al., 2000; Booth et al., 2002). Some reports claimed that fish fed extruded diets had a lower growth performances (Hilton et al., 1981; Booth et al., 2000)and/or did not affect growth (Aksnes et al., 1997; Venou et al., 2009).

Grass carp is a species of great importance for China aquaculture, and constitutes 7.18% of the world aquaculture production (FAO, 2010). Lysine levels can not satisfy the requirements in the commercial diets when plant protein meal is used as a major protein resource. Lysine deficiency inhibits the growth performance of grass carp and reduces the feed conversion efficiency (Wang et al., 2005). It is therefore necessary to balance the amino acid profile according to the supplementation of crystalline lysine in practical diets of grass carp. In China, most of cultured fish are fed extruded or pelleted diet. But the effects of extruded and pelleted diets fed by grass carp have not been studied. The aim of this study is to determine whether the processing method of feeds influences apparent digestibility, growth performance, serum biochemical parameters and dietary crystalline lysine utilization of grass carp.

Methods and material

Experimental diets and processing methods. Six practical diets were prepared (Table 1). The basal diet contained the minimum level of lysine, 13g kg-1 DM, from Soybean meal, Canola meal, Cotton meal, Rice bran meal and Wheat flour (Zhuhai Shihai Feed Corporation Ltd, Zhuhai, China). Lysine concentration was increased in two steps of approximately 2g lysine kg-1 Dry Matter (DM) each, with lysine originating from L-lysine HCL (78% L-lysine, CJ Co., Ltd, Liaocheng China).

The maximum intended lysine concentration was 17 g kg-1 DM, which is the optimally required by grass carp (Wang et al., 2005). Lysine supplementation was done by substituting L-Glutamic acid with L-lysine HCL. DL-methionine hydroxy analogue calcium (MHA-Ca, 84%,Novus International Inc. Zhibo, China) were supplied to meet Methionine requirement of grass carp. Amino acid composition of experimental diets is shown in Table 2. All diets were isoenergetic.

All dry ingredients were finely ground, weighed, mixed manually for 5 min and then transferred to a Hobart mixer (A-200T Mixer Bench Model unit, Resell Food Equipment Ltd, Ottawa, Canada) for another 15-min mixing. Soya lecithin was added to a pre-weighed premix of soy oil, and mixed until turned homogenous. The oil mix was then added to the Hobart mixer slowly while mixing was still continuing.

All ingredients were mixed for another 10 min after which distilled water was added (about 30% of diets) to the dough. Diets were manufactured in a pelletizer (Institute of Chemical Engineering, South China University of Technology, Guangzhou, China) and pelleted through a 1.5-mm die. The processing conditions were as follows: 100 rpm speed screw, 110℃ temperature and 30-45 atm pressure. All the diets were dried with forced air at 20℃ for 24 h until the moisture was reduced to about 10%. The dry feeds were placed in plastic bags and stored in a deep freezer at -20℃ until used.

Table 1 Formulation and approximate composition of practical diets for grass carp

	Extruded			Cold Pelleted		
Lysine level g kg^{-1}	13	15	17	13	15	17
Ingredient						
Soybean meal[a]	30	30	30	30	30	30
Canola meal[a]	320	320	320	320	320	320
Cotton meal[a]	200	200	200	200	200	200
Rice bran meal[a]	186.5	186.5	186.5	186.5	186.5	186.5
Wheat flour[a]	205	205	205	205	205	205
Glutamate acid [a]	5.2	2.6	0	5.2	2.6	0
Mineral mix[b]	5	5	5	5	5	5
Vitamin mix[c]	5	5	5	5	5	5
Soy oil[a]	5	5	5	5	5	5
Choline chlorine(50%)[a]	2	2	2	2	2	2
Monocalcium phosphate[a]	20	20	20	20	20	20
78%Lysine-HCL [d]	0	2.6	5.2	0	2.6	5.2
84%MHA-Ca [e]	5.2	5.2	5.2	5.2	5.2	5.2
Y_2O_3[f]	0.1	0.1	0.1	0.1	0.1	0.1
Phospholipid[a]	10	10	10	10	10	10
VC Ascorbic acid	1	1	1	1	1	1
Total	1000	1000	1000	1000	1000	1000
Proximate analysis (g kg^{-1})						
Moisture	63.3	59.9	67.7	77.2	76.8	81.7
Crude protein	310.7	311.9	312.9	309.5	308.6	307.8
Crude fat	31.5	31.1	31.5	32.1	31.8	31.8
Ash	76.8	76.7	76.6	75.5	75.5	75.5
Gross energy (KJ g^{-1})	4402	4375	4400	4500	4485	4480

[a]Zhuhai Shihai Feed Corporation Ltd, Zhuhai, China.
[b]Mineral mix (mg kg-1 of diet): MgSO4.7H2O, 315; ZnSO4.7H2O, 285; CaHPO4.2H2O, 250; FeSO4.7H2O, 200; MnSO4.H2O, 25; CoSO4.7H2O, 25; CaIO3, 25; CuSO4.5H2O, 15; Na2SeO3,10., (Guangzhou Chengyi Aquatic Technology Ltd, Guangzhou, China)
[c] Vitamin mix (mg kg-1 of diet): thiamin, 3; riboflavin, 8; vitmin A, 1 500IU; vitamin E, 40; vitamin D3, 2 000 IU; menadione, 6; pyridoxine, 4; cyanocobalamin, 2; biotin, 2; calcium pantothenate, 25; folic acid, 2; niacin, 12; inositol, 50. (Guangzhou Chengyi Aquatic Technology Ltd, Guangzhou, China)
[d] L-Lysine.HCL contained L-Lysine ≥ 78% (CJ Co., Ltd., Liaocheng, China)
[e] MHA-Ca contained DL-HMTBA (2-hydroxy-4-methylthio butanoic acid) ≥ 84% (Novus International Inc. Zhibo, China)
[f]Y2O3(Yttrium oxide), analytical pure (Weibo Chemical Ltd, Guangzhou, China)

Experimental layout. Grass carp juveniles from our facilities with an individual weight of 4.13±0.02 g were used. Before the experiment, the fish were acclimated to the experimental conditions for 2 weeks and fed to satiation with a commercial diet containing 30% protein and 4% lipid. Thirty healthy fish were randomly distributed to each of 18 experimental fiberglass tanks (98 L×48 W×42 H cm, water volume of 200 L) connected to a recirculation system. The water was oxygenated, and passed through an artificial sponge (3 cm thickness), coral-sand (25 cm thickness) and active-carbon filter (25 cm thickness) to remove chlorine. During the trial period, the diurnal photoperiod was 12-h light/12-h dark. Water quality parameters were monitored weekly as follows: temperature, 26.0±1.3℃; pH, 7.42±0.07, dissolved oxygen, 6.48±0.11 mg L-1; total ammonia-nitrogen, 0.45±0.02 mg L -1.

The fish were fed manually thrice with 5% body weight per day for 8 weeks. Feces were collected daily during the last 2 weeks. Feces from each tank were dried at 105 ℃ and stored at -70℃ for determination of digestibility with Y2O3 as indicator.

Table 2 Amino acid composition of experimental diets for grass carp (g kg-1 dry diets)

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Extruded				Cold Pelleted		
Essential amino acids g kg-1						
Lysine	13.2	15.1	16. 5	13	15	16.8
Methionine	10.9	11.3	10.7	10.3	9.9	9.7
Phenylalanine	13	12.7	13.9	11.6	12.3	12
Histidine	5.85	6.52	6.8	6.17	5.99	6.16
Tryptophan	0	0	0	0	0	0
Arginine	21.5	22.1	22.3	21	20.5	20.4
Threonine	9.53	9.82	9.92	9.07	8.95	9.26
Isoleucine	10.6	11.7	11	11	11.3	11.2
Leucine	17.6	18.6	19	19	19	19.1
Valine	15.3	15	15.4	14.8	14.7	14.7
Non-essential amino acids g kg-1						
Serine	8.96	9.7	9.56	8.37	8.38	8.71
Proline	13.7	13.1	13. 9	13.3	14.9	13.3
Cystine	1.67	1.66	1.67	1.67	1.65	1.65
Tyrosine	6.01	5.81	7.39	5.9	5.39	5.77
Aspartic acid	23.1	23	23.8	23	22.1	22.9
Glutamic acid	53.2	52.4	50.2	55.9	53.4	52.5
Glycine	13	13.5	13.2	13	12.6	13
Alanine	11.7	12.3	12.7	12.4	12.1	12.3
ΣAA	249	254	258	249	248	249

Sampling and analytical methods. At the beginning of the feeding trial, 18 fish were randomly sampled from the initial stock and sacrificed for analyses of whole body composition. At the end of the 56-day experiment, 10 fish from each tank were randomly collected for proximate analysis, 4 for analysis of whole-body composition and 6 were anaesthetized with tricaine methane sulphonate (MS222) (50 mg L-1) for blood collection individual weight whole body, viscera, liver and intraperitoneal fat as well as samples of white muscle from both sides of the skinless fillets were. The liver was dissected and frozen immediately in liquid nitrogen and stored at -70℃ until used. The serum was separated by centrifugation and stored at -70℃ until analyzed. Diets and fish samples (including white muscle and liver) were analyzed in triplicate for proximate composition. Crude protein, crude lipid, moisture, crude ash and gross energy (GE) were determined following standard methods (AOAC, 1984). Crude protein (N×6.25) was determined by the Kjeldahl method after acid digestion using an Auto Kjeldahl System (1030-Auto-analyzer, Tecator, Sweden). Crude lipid was determined by the ether extraction method using a Soxtec System HT (Soxtec System HT6, Tecator, Sweden). Moisture was determined by oven-drying at 105℃ for 24 h. Crude ash by incineration in a muffle furnace at 550℃ for 24h. Gross energy was determined using an adiabatic bomb calorimeter. Amino acids were analyzed following acid hydrolysis using high pressure liquid chromatography (HPLC; Hewlett Packard 1090, Palo Alto, USA). The concentrations of dietary and fecal Y2O3 were determined by inductively coupled serum atomic emission spectrophotometer (ICP; model: IRIS Advantage (HR), Thermo Jarrel Ash Corporation, Boston, U.S.A) after perchloric acid digestion (Bolin et al., 1952). The concentrations of Total serum protein (TP), Albumin (ALB), Cholesterol (CHO), Triacylglycerol (TG), glucose (GLU), Aspartate aminotransferase (AST), alanine aminotransferase (ALT), High density lipoprotein (HDL), Low density lipoprotein (LDL), Urea-N and Glutamate dehydrogenase

(GDH) were determined using an automatic blood analyser (Hitachi 7170A, Japan) from a clinical laboratory. Calculations and statistical analysis, apparent digestibility coefficients (ADCs) for dry matter, crude protein, and energy in the diets were determined with the following equations (Cho and Kaushik, 1990):

ADC of nutrients or energy %= 100 × [1−(Y2O3 in diet / Y2O3 in feces) × (% nutrient in feces / % nutrient in diet)]

The indexes for the assessment of growth performance were calculated as follows: Weight gain (WG , %)= 100 × (Wt - W0) / W0 Specific growth rate (SGR,% day-1) = (Ln Wt−LnW0) × 100 / 56

Where, W0 is the initial weight and Wt is the final weight Survival =100 × Final number/Initial number

Feed conversion ratio (FCR) = dry feed fed (g) / Wet weight gain (g)

Nitrogen retention (NR) =100×retained nitrogen (g) / nitrogen fed (g)

Protein efficiency ratio (PER) = Wet weight gain (g) / protein intake (g)

Lipid retention (LR) =100×retained lipid(g) / lipid fed (g)

Viscerasomatic index (VSI) =100×viscerasomatic weight (g) / body weight (g) Hepatopancreasomatic index (HSI) = 100×liver weight (g) / body weight (g) Intraperitoneal fat ratio (IPF) = 100×intraperitoneal fat weight (g) / body weight (g) Condition factor (CF) = 100×body weight (g) / body length (cm)3.

All data are presented as Means ± SEM. The SPSS software Ver. 13.0 for Windows of GLM procedure (SPSS Inc., Chicago, IL, USA Ver13.0, USA) was used to conduct factorial ANOVA. When interaction between processing method and amino acid supplementation was statistically significant for a particular response, differences among lysine levels within each diet type were determined using Tukey's mean separation. Treatment effects and interactions were considered significant at $P < 0.05$.

Results Fish readily accepted the experimental diets and survival rate were very high during the 8 weeks feeding trial. Growth performances and nutrition retention of grass carp are presented in Table 3.

Table 3 Effect ㎜ growth and nutrition retention of grass carp with supplemental lysine in different processing methods

	Extruded			Cold pelleted			*Two-Way Anova*		
Lysine_1evel g kg-1	13	15	17	13	15	17	PM	Lys	PM- Lys
Initial Body Weight (g)	4.14±0.01	4.13±0.01	4.11±0 .01	4.13±0.01	4.11±0.01	4.14±0.01	0.657	0.327	0.149
Final Body Weight (g)	17.3±0.14	18.0±1.47	19.6±0.50	17.10±0.20	18.9±0.35	20.2±0.12	0.58	0.036	0.795
Weight gain (%)	319±6.34	336±3.27	377±1 1.5	314±2.59	360±9.03	387±2.71	0.555	0.032	0.766
Specific growth rate (% day-1)	2.560±02	2.620±14	2.79±0.04	2 .54±0.01	2.72±0.04	2.83±0.01	0.53	0.031	0.718
Surviva\(%)	98.3±1.67	98.9±1.11	100.0±0.0	98.3±l.67	97.8±1.11	97.8±1.11	0.302	0.875	0.704
Feed conversion ratio	1.561±0.03	1.521±0.14	1.31±0.04	1.59±0.01	1.371±0.01	1.281±0.02	0.436	0.021	0.528
Nitrogen retention	30.31±2.33	32.71±1.30	36.1±r0.31	31.11±1.12	35.5±l.11	38.71±0.42	0.072	0.002	0.708
Protein efficiency ratio	2.041±0.07	2.131±0.19	2.441±0.08	2.041±0.02	2.311±0.02	2.53±0.05	0.359	0.012	0.737
Lipid retention	184±7.78	167±7.02	190±2.84	157±0.39	152±10.8	145±13.4	0.006	0.552	0.316

There were no significant differences in survival among fish fed all the diets. After the feeding trial, final body weight (FBW), weight gain (WG), specific growth rate (SGR), nitrogen retention (NR) and protein efficiency ratio (PER) of grass carp significantly improved in relation to lysine inclusion ($P < 0.05$). Processing method had no effect on growth performance of fish. Lipid retention (LR) of fish fed extruded diet was significantly higher with than fish fed with pelleted diet ($P < 0.05$). Feed conversion ratio (FCR) was significantly improved in relation to lysine level ($P < 0.05$), and was not affected by feed processing method. Interaction was not found between feed processing method and the level of lysine. The proximate body composition (moisture, protein, and lipid) and the morphometry index of grass carp are shown in Table 4. Lipid content of the whole body and muscle significantly increased when fish were fed with the extruded diets ($P < 0.05$). Viscerasomatic index (VSI), hepatopancreasomatic index (HSI) and intraperitoneal fat ratio (IPF) decreased with increasing levels of dietary lysine ($P < 0.05$) and were

significantly higher than those fed the cold pelleted diets ($P < 0.05$). There were no interactions between body composition and morphometry index between feed processing method and lysine level.

Table 4 Body composition and morphometry index of grass carp fed experimental diets for 56 days

	Extruded			Cold Pelleted			*Two-way ANOVA*		
Lysine level g kg^{-1}	13	15	17	13	15	17	PM	Lys	PM×Lys
Whole body Composition (g kg^{-1}) [1]									
Moisture	734±0.71	736±7.41	744±2.11	738±5.52	745±2.22	747±1.68	0.198	0.190	0.805
Protein	146±5.01	151±5.58	146±2.61	149±5.22	150±2.83	150±3.66	0.567	0.782	0.853
Lipid	88.9±5.22	78.9±2.77	78.8±1.11	79.8±0.73	70.6±3.32	66.5±5.84	0.017	0.055	0.873
Ash	26.6±0.72	27.9±0.53	28.1±0.14	28.5±0.22	30.6±0.64	30.2±0.89	0.004	0.079	0.840
Muscle									
Moisture	794±1.82	798±3.11	798±2.24	795±3.01	798±1.69	798±0.44	0.839	0.145	0.987
Protein	180±0.44	182±7.88	175±4.11	185±2.75	181±1.14	182±0.14	0.448	0.562	0.726
Lipid	14.7±1.81	12.8±0.84	10.7±0.89	10.7±0.28	10.6±0.58	9.02±0.89	0.009	0.049	0.527
Liver									
Moisture	595±32.9	607±19.2	611±10.2	610±11.7	635±13.8	635±7.41	0.152	0.478	0.940
Protein	105±6.72	106±6.41	107±3.32	101±6.04	110±6.57	114±3.72	0.657	0.515	0.675
Lipid	174±21.6	178±13.1	158±8.2	180±30.5	134±8.41	159±4.02	0.186	0.619	0.318
Morphometry [2]									
Viscerasomatic index	8.75±0.21	8.09±0.19	7.81±0.17	7.40±0.20	7.38±0.22	7.22±0.14	0.000	0.019	0.122
Hepatopancreasomatic index	2.06±0.10	1.94±0.08	1.77±0.06	1.87±0.11	1.71±0.09	1.62±0.06	0.008	0.008	0.871
Intraperitoneal fat ratio	2.89±0.25C	2.47±0.11	1.97±0.14	2.38±0.15	1.95±0.09	1.85±0.09	0.001	0.000	0.238
Condition factor	2.00±0.03	1.96±0.04	1.94±0.03	2.04±0.03	1.98±0.02	1.99±0.03	0.124	0.177	0.931

Means±S.E.M. of three replicates.
PM: Processing method; Lys: Lysine

Apparent digestibility of dry matter, protein, and energy are given in Table 5. Energy apparent digestibility significantly increased by extrusion ($P < 0.05$), but not with the dietary lysine level. Processing method and dietary lysine level did not have any significant effects on ADC values of dry matter and protein. No interaction was detectable between the two experimental factors with regard to digestibility. Serum biochemical parameters are provided in Table 6. LDL content was significantly reduced with increasing levels of dietary lysine level ($P < 0.05$). While AST and GDH of grass carp fed extruded diets were significantly lower than fed those fed with cold pelleted diets ($P < 0.05$). Serum HDL and LDL of grass carp fed extruded diets were significantly higher than fed those fed with cold pelleted diets showed an opposite trend ($P < 0.05$). There were no significant differences in Serum Urea-N.

Table 5 Apparent digestibility of nutrients and energy of juvenile grass carp fed with lysine supplementation in different processing methods

	Extruded			Cold pelleted			*Two-Way Anova*		
Lysine level g kg^{-1}	13	15	17	13	15	17	PM	Lys	PM-Lys
Dry matter (%)	78.3±0.32	78.7±0.15	78.9±0.12	78.6±0.68	18.6±0.64	78.8±0.60	0.845	0.286	0.653
Protein (%)	92.3±0.36	92.6±0.23	92.7±0.21	92.7±0.10	92.5±0.45	93.0±0.17	0.225	0.296	0.382
Energy (%)	83.6±0.47	83.7±0.29	83.6±0.50	82.0±0.72	82.3±0.49	82.1±0.21	0.002	0.269	0.901

Means±S.E.M. of three replicates, Probability associated with the F statistic for the factorial ANOVA
PM: Processing method;
Lys: Lysine

Table 6 Biochemical compositions of serum from grass carp with supplemental lysine in different processing methods

	Extruded			Cold Pelleted			*Two-way ANOVA*		
Lysine level g kg^{-1}	13	15	17	13	15	17	PM	Lys	PM×Lys
Aspartate aminotransferase u l^{-1}	21.5±1.15	30.0±1.35	29.3±1.61	35.0±4.90	31.9±1.13	38.6±4.76	0.006	0.189	0.202
Alanine aminotransferase u l^{-1}	3.03±0.29	2.25±0.35	2.60±0.35	2.15±0.15	2.60±0.60	3.07±0.47	0.952	0.651	0.296
Total serum protein g L^{-1}	25.2±1.54	24.0±1.54	22.9±1.29	22.3±2.40	24.2±1.24	22.2±0.88	0.359	0.536	0.590
Albumin g L^{-1}	9.07±1.19	8.93±1.56	9.47±0.37	7.50±1.50	8.77±0.67	8.53±0.67	0.325	0.791	0.810
Cholesterol mmol l^{-1}	5.43±0.36	5.16±0.20	4.81±0.29	4.28±0.61	4.63±0.24	4.19±0.16	0.101	0.373	0.583
Triacylglycerol mmol l^{-1}	2.79±0.25	2.21±0.33	2.02±0.20	2.16±0.18	2.33±0.20	2.10±0.05	0.440	0.226	0.227
High density lipoprotein mmol l^{-1}	1.21±0.09	1.20±0.28	1.17±0.07	1.01±0.15	1.11±0.04	1.03±0.05	0.029	0.658	0.755
Low density lipoprotein mmol l^{-1}	1.31±0.14	1.17±0.05	0.98±0.12	1.06±0.12	0.90±0.09	0.73±0.03	0.012	0.028	0.995
Glucose mmol l^{-1}	7.40±0.70	8.60±0.75	8.10±0.77	9.00±0.30	8.87±1.44	9.63±0.57	0.151	0.756	0.704
Urea-N u l^{-1}	0.80±0.26	0.93±0.08	0.93±0.33	1.20±0.40	1.27±0.08	1.07±0.03	0.057	0.783	0.713
Glutamate dehydrogenase u l^{-1}	5.67±2.02	7.00±2.65	10.0±0.57	14.0±0.00	10.7±2.33	14.0±0.57	0.005	0.234	0.424

Means±S.E.M. of three replicates.
PM: Processing method; Lys: Lysine

Discussion

In this study, two tested feed processing methods had no effect on growth performance and FCR. This is in accordance with other reports (Aksnes et al 1997). Some researchers reported that rainbow trout and Bidyanus bidyanus fed extruded diets always had a lower voluntary intake than fed steamed pelleted diets and consequently had lower weight gain (Booth et al., 2000 ; Booth et al., 2002 ; Hilton et al., 1981). The fish in each treatment were fed with the same quantity of feed (5% body weight) per day and there was no difference of feed intake among each treatment. There is a controversy on the utilization of crystal amino acids by fish. Most studies show that crystalline amino acids are utilized efficiently and meet EAA requirements of fish (Viola,1991; Mukhopadhayay, 1999 ; Sardar et al., 2009). In our study, the growth performance and FCR of grass carp fed with pelleted and extruded diet were significantly improved with lysine-HCL supplementation, indicating that grass carp can utilize crystalline amino acids. Grass carp fed extruded diets had a higher IPF, VSI, HIS and lipid content of body than fed cold pelleted diets. Extrusion processing also significantly increased body lipid content of Sparus aurata (Aksnes et al., 1997 ; Venou et al., 2009). HDL and LDL content of serum were significantly increased with the extruded diet in our study. All these results were probably achieved by improvement of energy apparent digestibility of the extruded feeds. With supplementation of crystalline lysine in diet, IPF, VSI, HIS and body lipid content of fish were reduced in both of the tested diets; these reflect the proportional accumulation of energy in both the abdominal cavity and the liver. It has been widely acknowledged that feeding diets deficient in amino acid result in excess energy deposition as fat in the liver, fillet or abdominal cavity (Brown, 1992). Lysine is a precursor of L-carnitine which has an important function in lipid and energy metabolism of fish (Santulli & D'Amelio, 1986). The major metabolic role of L-carnitine is to transport long-chain fatty acids into the mitochondria for β-oxidation. Deficiency of carnitine impairs therefore energy metabolism and membrane function (Harmeyer, 2002), and reduces the amount of long-chain fatty acids available for storage in adipose tissues. In the present study, protein sources of the experimental diets were from cotton meal, soybean meal and canola meal, those plants protein resources that are known to include anti-nutrients (Gatlin III et al., 2007). Serum AST and GDH activity of fish fed extruded diets had significantly lower than fed cold pelleted diets. High Serum AST and GDH generally indicate the damage or weakening of normal liver function. Serum AST and GDH were often considered as markers of hepatocellular injury (O'Brien et al., 2000; Pan et al., 2010). Extrusion may be helpful to reduce anti-nutrients content in plant-based practical diets.

Conclusions

The results of the present study showed processing method had no effect on growth performance and lysine utilization of grass carp. The growth performance of grass carp

was significantly improved when fed diet with crystalline lysine supplementation. Acknowledgments We thank Y.J. Gao, J.Y. He and Y. Jin for sample collection. The work was financially supported by the fund of National Natural Science Foundation of China (31202007) and national modern industrial technology system of shrimp（nycytx46）and effective environmental protection feed of fish and shrimp (2007BAD29B04).

References

Akimoto A, Takeuchi T, Satoh S, Watanabe T, 1992. Effect of extrusion processing on nutritional value of brown fish meal diets for rainbow trout. *Bulletin of the Japanese Society of Scientific Fisheries*, 58: 1477-1482.

Aksnes A, Izquierdo M, Robaina L, Vergara J, Montero D, 1997. Influence of fish meal quality and feed pellet on growth, feed efficiency and muscle composition in gilthead seabream (*Sparus aurata*). *Aquaculture*, 153: 251-261.

AOAC ,1984. *Official Methods of Analysis*,14th edn, Arlington, VA.

Bolin DW, King RP, Klosterman EW,1952. A simplified method for the determination of chromic oxide when used as an index substance. *Science*, 116: 634-635.

Booth M, Allan GL, Evans AJ, Gleeson VP, 2002. Effects of steam pelleting or extrusion on digestibility and performance of silver perch *Bidyanus bidyanus*. *Aquaculture Research*, 33: 1163-1173.

Booth MA, Allan GL, Warner-Smith R, 2000. Effects of grinding, steam conditioning and extrusion of a practical diet on digestibility and weight gain of silver perch, *Bidyanus bidyanus*. *Aquaculture*, 182: 287-299.

Brown ML, Nematipour, G.R., Gatlin, D.M, 1992. Dietary Protein Requirement of Juvenile Sunshine Bass at Different Salinities. *The Progressive Fish-Culturist*, 54:148-156.

Buser MD, Abbas HK, 2001.Mechanically processing cottonseed to reduce gossypol and aflatoxin levels. *Toxin Reviews*, 20:179-208.

FAO, 2010. 2008 FAO year book annuaire:Fishery and aquaculture statistics. *FAO*, Rome, pp. 28.

Gatlin III DM, Barrows FT, Brown P, Dabrowski K, Gaylord TG, Hardy RW, Herman E, Hu G, Krogdahl, Nelson R, 2007. Expanding the utilization of sustainable plant products in aquafeeds: a review. *Aquaculture Research*, 38: 551-579.

Harmeyer J, 2002. The physiological role of L-carnitine. *Lohmann information*, 27: 15-22.

Hilton J, Cho C, Slinger S, 1981. Effect of extrusion processing and steam pelleting diets on pellet durability, pellet water absorption, and the physiological response of rainbow trout (*Salmo gairdneri* R.). *Aquaculture*, 25: 185-194.

Misra CK, Sahu N, Jain K, 2002. Effect of extrusion processing and steam pelleting diets on pellet durability, water absorption and physical response of *Macrobrachium rosenbergii*. *Asian-Australasian Journal of Animal Sciences*, 15: 1354-1358.

Mukhopadhayay NR, A.K., 1999. Improvement of quality of sal (*Shorea robusta*) seed meal protein with supplemental amino acids in feed for rohu, *Labeo rohita* (Hamilton), fingerlings. *Acta Ichthyol Fiscal*, 29: 25-39.

O'Brien P, Slaughter M, Swain A, Birmingham J, Greenhill R, Elcock F, Bugelski P, 2000. Repeated acetaminophen dosing in rats: adaptation of hepatic antioxidant system. *Human and experimental toxicology*, 19: 277-283.

Pan CH, Chien YH, Wang YJ, 2010. The antioxidant capacity response to hypoxia stress during transportation of characins (*Hyphessobrycon callistus* Boulenger) fed diets supplemented with carotenoids. *Aquaculture Research*, 41: 973-981.

Santulli A, D'Amelio V ,1986. Effects of supplemental dietary carnitine on growth and lipid metabolism of hatchery-reared sea bass (*Dicentrarchus labrax* L.). *Aquaculture*, 59, 177-186.

Sardar P, Abid M, Randhawa HS, Prabhakar SK, 2009. Effect of dietary lysine and methionine supplementation on growth, nutrient utilization, carcass compositions and haemato-biochemical status in Indian Major Carp, Rohu (*Labeo rohita* H.) fed soy protein-based diet. *Aquaculture Nutrition*, 15: 339-346.

Satoh S, Higgs D, Dosanjh B, Hardy R, Eales JG, Deacon G, 1998. Effect of extrusion processing on the nutritive value of canola meal for chinook salmon (*Oncorhynchus tshawytscha*) in seawater. *Aquaculture Nutrition*, 4: 115-122.

Venou B, Alexis M, Fountoulaki E, Haralabous J, 2009. Performance factors, body composition and digestion characteristics of gilthead sea bream (*Sparus aurata*) fed pelleted or extruded diets. *Aquaculture Nutrition*, 15: 390-401.

Vens-Cappell B, 1984. The effects of extrusion and pelleting of feed for trout on the digestibility of protein, amino acids and energy and on feed conversion. *Aquacultural engineering*, 3: 71-89.

Wang S, Liu Y, Tian L, Xie M, Yang H, Wang Y, Liang G, 2005. Quantitative dietary lysine requirement of juvenile grass carp *Ctenopharyngodon idella*. *Aquaculture*, 249: 419-429.

Yoshitomi B, 2004. Effect of extrusion cooking temperature on the microstructure of extruded pellets. *Fisheries Science*, 70: 1157-1163.

Viola S, Lahav E ,1991. Effects of lysine supplementation in practical carp feeds on total protein sparing and reduction of pollution. *Isr. J. Aquacult. - Bamidgeh*, 43: 112-118.